EXHIBIT "A"

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of DENSLOW et al.	Confirmation No: 3958
Application No. 10/663,561	Examiner: SALMON, Katherine. D.
Filed: September 15, 2003	Group Art Unit: 1634

For: DETECTING HORMONALLY ACTIVE COMPOUNDS

37 C.F.R § 1.132 DECLARATION

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Nancy D. Denslow, PhD, declare as follows:

1. I am one of the named inventors and am familiar with patent application No. 10/663,561 entitled " DETECTING HORMONALLY ACTIVE COMPOUNDS " (hereafter the '561 application) and the subject matter described therein.

2. I hold a PhD degree in Biochemistry and Molecular Biology and currently am working in environmental toxicology. I am presently an Associate Professor of Physiological Sciences at the University of Florida, Gainesville, Florida.

3. I have authored or coauthored 110 scientific papers, and two issued patents (Patent #5,650,299 -- Stem Cell Proliferation Factor, Michael Lawman, Pat Lawman and Nancy Denslow.

July 22, 1997; Patent #5,981,708 – Stem Cell Proliferation Factor, Michael Lawman, Pat Lawman and Nancy Denslow. November 9, 1999).

4. I have reviewed the Final Office Action dated September 5, 2006. I have been asked by patent counsel Zachariades to provide an explanation based on the claimed invention showing that the subject matter of the claims is not restricted to sheepshead minnow and largemouth bass fish but can be applied to other fish species without undue experimentation, unpredictability or requirement of high level of skill.

Claim 1 is copied below:

Claim 1. A method for detecting the presence of an agent having estrogenic or androgenic activity in a sample, the method comprising the steps of:

(A) providing at least one fish cell which was exposed to the sample;

(B) analyzing the at least one fish cell for expression of at least one gene wholly or partially encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO's: 146, 148, 149, 166, 167, 178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529 for identifying estrogen activity and SEQ ID NO's: 14, 15, 25, 28, 30, 42, 44, 47, 52, 61, 62, 71, 558 and 555 for identifying androgenic activity; and

(C) comparing the expression of the at least one gene in the cell compared to the expression of the at least gene in a control cell not exposed to the sample or an agent having estrogenic or androgenic activity, wherein a difference in the expression of the at least one gene in the at least one fish cell compared to the expression of the at least one gene in the control cell indicates that the sample contains an agent having estrogenic or androgenic activity.

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5. The Examiner rejects claims 1-7 and 10-32 are rejected in the instant Application in the September 5, 2006 Office Action " because the claimed invention does not reasonably provide enablement for any fish species or detection of genes partially encoded. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims."

6. I will address the Examiner's comments regarding the scope of enablement of the instant invention.

7. We describe methods of detecting expression of genes in response to agents which cause estrogenic or androgenic activity in a sample.

As discussed previously, the endocrine systems of fish are highly conserved and work by essentially the same pathways. In all fish, estrogens and androgens bind to their respective receptors to cause them to dimerize and then bind to promoter regions on genes that they control. This binding activates transcription of this set of genes. Estrogen and androgen receptors are highly conserved in sequence (for example among fish species the homology can extend up to 90%) and they regulate the same set of specific genes in different species of fish. So, that it is reasonable to expect that the same set of genes would be regulated by estrogen (and estrogen mimics) or androgen (and androgen mimics) in sheepshead minnows and largemouth bass as in

other fish. As such, by identifying the name of the gene in these species, one of ordinary skill in the art can identify these genes in other species based on the teachings of the specification. The step-by-step methodology that can be utilized by anyone of ordinary skill in the art is based on the teachings of the invention:

Method to Identify Homologous Sequences in other Fish Species

1. The DNA sequence for the biomarkers we have identified in largemouth bass or in sheepshead minnow can be translated to the protein sequence by using a translation program, for example the one at the following url: http://us.expasy.org/tools/dna.html

2. This program will translate the DNA segment in all 6 reading frames. The correct reading frame is selected by looking for an open reading frame (between stop codons). Because the sequences were obtained by several methods, the correct reading frame may be in any one of the 6 reading frames. The correct reading frame is confirmed by using the program BLAST P that compares protein sequence to the protein databases. This program is available at the following url: http://www.ncbi.nlm.nih.gov/blast/ and using the BLASTP program in the protein box.

3. The results of the BLAST P program identify the gene in question for all other fish species that have been deposited in gene bank, so far. The fact that genes in mammals also are identified suggests that the equivalent gene is probably in all vertebrate species.

4. If the top result is a reference to an EST in pufferfish, then one can double click the entry and then go to BLINK which will show the closest match to this EST in the entire database. Often this will show the actual name of the protein and that it is identified in multiple fish species.

5. Table 1 contains the DNA sequences and predicted protein sequences for the abbreviated list of biomarkers in the patent.

6. Fig. 1 contains examples of doing this search for three of the biomarkers on the list:

    A. ER alpha
    B. StAR
    C. Spermidine/spermine acetyl transferase

Fig. 1. Example of output from blast for two biomarkers

A. ER Alpha Sequences producing significant alignments:	Score (Bits)	E Value
gi|42475913|gb|AAG44622.2|AF253062_1 estrogen receptor alpha [Mi	1077	0.0
gi|51100569|emb|CAD43599.1| oestrogen receptor alpha [Dicentrarc	958	0.0
gi|3122078|sp|O42132|ESR1_PAGMA Estrogen receptor (ER) (Estra...	869	0.0
gi|28192419|gb|AAL82743.1| estrogen receptor alpha [Acanthopagru	840	0.0
gi|115313958|gb|AAD31032.2|AF136979_1 estrogen receptor type alp	833	0.0
gi|85013465|gb|ABC68615.1| estrogen receptor alpha [Kryptolebias	831	0.0
gi|18996336|dbj|BAB85622.1| estrogen receptor alpha [Paralichthy	831	0.0
gi|12643248|sp|Q9PVZ9|ESR1_SPAAU Estrogen receptor (ER) (Estr...	822	0.0
gi|109804224|emb|CAK95869.1| estrogen receptor type I [Oreochrom	822	0.0
gi|3915675|sp|P50241|ESR1_ORYLA Estrogen receptor (ER) (Estra...	818	0.0
gi|31158286|dbj|BAC76957.1| estrogen receptor a [Fundulus hetero	816	0.0
gi|12230057|sp|Q9YH33|ESR1_ORENI Estrogen receptor (ER) (Estr...	815	0.0
gi|47224612|emb|CAG03596.1| unnamed protein product [Tetraodon n	812	0.0
gi|32186922|gb|AAP72178.1| estrogen receptor alpha [Halichoeres	810	0.0
gi|29169337|gb|AAO66473.1| estrogen receptor alpha [Zoarces vivi	809	0.0
gi|40217924|gb|AAR82891.1| estrogen receptor alpha [Astatotilapi	795	0.0
gi|2507414|sp|P50240|ESR1_OREAU Estrogen receptor (ER) (Estra...	783	0.0
gi|60101766|gb|AAX13999.1| estrogen receptor alpha [Oryzias java	783	0.0
gi|112982639|dbj|BAF03498.1| estrogen receptor alpha [Kryptolebi	781	0.0
gi|50293047|gb|AAT72914.1| estrogen receptor alpha [Fundulus het	780	0.0
gi|86278355|gb|ABC88430.1| estrogen receptor alpha short form [K	750	0.0
gi|12585224|sp|P57753|ESR1_MICUN Estrogen receptor (ER) (Estr...	746	0.0
gi|74422193|gb|AAY25396.3| estrogen receptor alpha [Salmo salar]	694	0.0
gi|82582235|gb|AAS92970.2| estrogen receptor alpha [Oncorhynchus	692	0.0
gi|12643267|sp|P16058|ESR1_ONCMY Estrogen receptor (ER) (Estr...	692	0.0
gi|83026879|gb|ABB96483.1| estrogen receptor alpha [Pseudolabrus	686	0.0
gi|5101849|emb|CAB45140.1| estrogen receptor [Oncorhynchus mykis	680	0.0
gi|77020881|gb|ABA60432.1| estrogen receptor alpha 2 [Oncorhynch	651	0.0
gi|12061010|gb|AAG48341.1|AF326201_1 estrogen receptor [Halichoe	629	2e-178
gi|1706708|sp|P50242|ESR1_SALSA Estrogen receptor (ER) (Estra...	628	3e-178
gi|103903|pir||A37197 estrogen receptor - rainbow trout	609	2e-172
gi|82409100|gb|ABB73308.1| estrogen receptor alpha 1 [Oncorhynch	595	2e-168
gi|16118451|gb|AAL12298.1| estrogen receptor alpha [Carassius au	593	9e-168
gi|56692005|emb|CAD32175.1| putative estrogen receptor alpha [Ca	592	1e-167
gi|61097788|dbj|BAD91035.1| estrogen receptor alpha [Rutilus rut	585	2e-165
gi|84619480|emb|CAD67996.1| putative estrogen receptor alpha ...	583	6e-165
gi|52789059|gb|AAU87498.1| estrogen receptor alpha [Pimephale...	577	9e-163
gi|23308675|ref|NP_694491.1| estrogen receptor 1 [Danio rerio...	575	2e-162
gi|95115499|gb|ABF56051.1| estrogen receptor alpha [Spinibarbus	575	3e-162
gi|10944302|dbj|BAB16893.1| estrogen receptor [Danio rerio]	574	5e-162
gi|13872679|emb|CAC37560.1| estrogen receptor alpha [Clarias gar	561	3e-158
gi|12230058|sp|Q9YH27|ESR1_ICTPU Estrogen receptor (ER) (Estr...	556	2e-156
gi|38327072|gb|AAR17610.1| estrogen receptor alpha-2 [Carassius	554	4e-156
gi|3818524|gb|AAC69548.1| estrogen receptor type alpha [Ictaluru	552	2e-155
gi|83316220|gb|ABC02394.1| estrogen receptor alpha [Hippoglossus	526	1e-147

B. StAR

Sequences producing significant alignments:

	Score (Bits)	E Value
gi|73915382|gb|AAZ92554.1| steroidogenic acute regulatory protei	498	2e-139
gi|109627819|gb|ABG34343.1| mitochondrial steroidogenic acute...	485	1e-135
gi|116090847|gb|ABJ56005.1| steroidogenic acute regulatory prote	448	2e-124
gi|20140240|sp|Q9DEB4|STAR_ONCMY Steroidogenic acute regulato...	445	1e-123
gi|21362963|sp|Q9DE06|STAR_SALFO Steroidogenic acute regulato...	442	1e-122
gi|29243194|dbj|BAC66210.1| steroidogenic acute regulatory prote	436	8e-121
gi|47209041|emb|CAF91743.1| unnamed protein product [Tetraodon n	421	2e-116
gi|18859431|ref|NP_571738.1| steroidogenic acute regulatory p...	420	4e-116
gi|31323270|gb|AAP44111.1| steroidogenic acute regulatory protei	416	7e-115
gi|31323272|gb|AAP44112.1| steroidogenic acute regulatory protei	415	1e-114
gi|89474611|gb|ABD73012.1| steroidogenic acute regulatory protei	413	5e-114
gi|21362964|sp|Q9DG08|STAR_XENLA Steroidogenic acute regulato...	378	2e-103
gi|63100238|gb|AAH95917.1| Unknown (protein for MGC:99207) [Xeno	376	7e-103
gi|83405128|gb|AAI10789.1| MGC131332 protein [Xenopus laevis]	373	6e-102
gi|86276864|gb|ABC87916.1| steroidogenic acute regulatory protei	366	6e-100
gi|30584323|gb|AAP36410.1| Homo sapiens steroidogenic acute r...	345	2e-93
gi|57097785|ref|XP_532807.1| PREDICTED: similar to steroidoge...	345	2e-93
gi|60833148|gb|AAX37038.1| steroidogenic acute regulator [synthe	345	2e-93
gi|56243551|ref|NP_000340.2| steroidogenic acute regulator is...	345	2e-93
gi|45382719|ref|NP_990017.1| steroidogenic acute regulator [G...	345	2e-93
gi|114619693|ref|XP_001170357.1| PREDICTED: steroidogenic acu...	345	2e-93
gi|727253|gb|AAC50141.1| steroidogenic acute regulatory prote...	344	3e-93
gi|109086164|ref|XP_001090472.1| PREDICTED: similar to steroi...	342	1e-92
gi|116242803|sp|Q28918|STAR_BOVIN Steroidogenic acute regulat...	340	6e-92
gi|3133060|emb|CAA76718.1| steroidogenic acute regulatory protei	337	6e-91
gi|1809329|gb|AAB41674.1| steroidogenic acute regulatory protein	336	7e-91
gi|27806397|ref|NP_776614.1| steroidogenic acute regulatory p...	335	1e-90
gi|3915025|sp|O46689|STAR_HORSE Steroidogenic acute regulator...	335	2e-90
gi|57163979|ref|NP_001009243.1| steroidogenic acute regulator...	335	2e-90
gi|34582612|gb|AAQ76091.1| steroidogenic acute regulatory protei	335	2e-90
gi|7514090|pir||JC5386 steroidogenic acute regulatory protein -	334	4e-90
gi|56711334|ref|NP_998920.2| steroidogenic acute regulatory p...	333	4e-90
gi|45439548|gb|AAS64369.1| steroidogenic acute regulatory pro...	333	8e-90
gi|2498964|sp|Q28996|STAR_PIG Steroidogenic acute regulatory ...	332	1e-89

C. Spermidine/spermine acetyl transferase

Sequences producing significant alignments:	Score (Bits)	E Value
gi|50344994|ref|NP_001002169.1| hypothetical protein LOC43171...	272	5e-72
gi|71834540|ref|NP_001025370.1| hypothetical protein LOC56570...	266	3e-70
gi|68404148|ref|XP_696280.1| PREDICTED: similar to spermidine...	256	3e-67
gi|45383744|ref|NP_989517.1| spermidine/spermine N1-acetyltra...	254	1e-66
gi|28188751|gb|AAO16805.1| spermidine/spermine N1-acetyltrans...	251	9e-66

The entire sequence or any part of the sequence (at least 30 nucleotides in length) that is unique to the gene (or homolog) would provide correlative expression levels between control and exposed cells. Unique segments for genes can be determined by testing any segment via BLASTN to the entire genome sequence. A few segments may be specific for gene families (rather than the specific gene mentioned) and these segments would have lower correlative value – for example the DNA binding domain of all estrogen receptors (alpha, beta and gamma) is 95% identical – a sequence containing only this domain would not distinguish the three receptors from each other but would give an average value for their expression.

The Examiner asserts on page 7, beginning on line 16: ."the specification does not teach if these genes are observed in other species of fish and whether these genes provide the same expression in those species." In this case, all three of the receptors are up regulated by estrogen. The genes are expressed in other species of fish – as can be determined by BLASTP of the genes in Table 1. The inference is that these genes would be changed by estrogen or androgen in all fish species. Research we have performed in the last couple of years indicates that homologs of these

same genes are changed in fathead minnows (a freshwater species) that are exposed to estrogens and androgens, among other genes.

Thus, by identifying the genes for largemouth bass or for sheepshead minnow that respond to stimulation by estrogens or androgens, either by increased or decreased expression, one can identify homologs in other fish species for which there is gene sequence information in the databases. Thus knowing that estrogen or androgen alter expression of this set of genes in largemouth bass and sheepshead minnow, one can reasonably expect that the same set of genes (or a subset of these genes) will respond in like manner in all fish species. The instant specification thus, allows one of ordinary skill in the art to identify homologs from any fish species without undue experimentation or otherwise.

8. Next, I will address some of the issues raised by the Examiner in the Office Action.

On page 8, beginning on line 3 of the Office Action: "This would require a large amount of inventive effort, with each of the many intervening steps, upon effective reduction to practice, not providing any guarantee of success in the succeeding steps."

Applicants disagree. There are microarrays commercially available for zebrafish and fathead minnow (each containing 60mer oligonucleotides) that could be used to determine response to estrogen or androgen. These experiments are straight forward and do generate data that corroborates the original statements that the genes in the instant application are altered by

exposure to estrogens and androgens. It is now possible to get abundant EST sequences from any fish species that would allow similar interpretation.

On Page 9 line 16 of the Office Action: "..... The search of the SEQ ID NOS claimed indicates homology is not identical among species of fish.....:

Homology does not need to be identical among species of fish. One would identify the genes in other species by using the BLASTP program – as more sequence information is available, these genes would be identifiable in all species. The BLASTP program shows homology with mammalian species as well, thus the similarity would extend to all vertebrates. The homology does not need to be > 90% -- genes are normally found to be homologs because they share high homology in blocks of sequences that are highly conserved and may have low homology in other regions which are of less importance. Thus, one should be able to still identify the homologs and then design specific oligonucleotides based on the sequence of the homologs to test the species of interest.

On page 10, line 11: "if the scientist used the wrong probe sequence within the vitellogenin gene to make a gene chip, there is a high probability that no positive response would be observed on the chips....."

The first step is to get sequence information for the gene of interest from any fish species. Then there is an algorithm that can be used to design the best 60'mer oligonucleotide for

application to an array (or one could use the entire cDNA or the fragment obtained). For oligonucleotide probes there is a correlation of better probes with position on the cDNA towards the 3'-end. The algorithm to design oligonucleotide probes is now available on the Agilent web page. However the probes are designed, prior to use, it would be important to run the BLASTN program to determine that the sequence maps to only one gene.

9. I further state that all statements made herein are of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with my knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Nancy Denslow
Dr. Nancy D. Denslow

JAN 4, 2007
Date